Local adaptation and embryonic plasticity affect antipredator traits in hatchling pond snails

Dalesman, Sarah; Thomas, Angharad; Rundle, Simon D.

Published in:
Freshwater Biology

DOI:
10.1111/fwb.12512

Publication date:
2015

Citation for published version (APA):
Dalesman, S., Thomas, A., & Rundle, S. D. (2015). Local adaptation and embryonic plasticity affect antipredator traits in hatchling pond snails. *Freshwater Biology*, *60*(4), 663-672. https://doi.org/10.1111/fwb.12512

Freshwater Biology

Freshwater Biology (2014) doi:10.1111/fwb.12512

Local adaptation and embryonic plasticity affect antipredator traits in hatchling pond snails

SARAH DALESMAN*, ANGHARAD THOMAS† AND SIMON D. RUNDLE‡

*Institute of Biological Environmental and Rural Sciences, Aberystwyth University, Aberystwyth, U.K.

†Biosciences, College of Life and Environmental Sciences, University of Exeter, Exeter, U.K.

‡Marine Biology and Ecology Research Centre, School of Marine Science and Engineering, University of Plymouth, Plymouth, U.K.

SUMMARY

1. The biology of induced defences in very early life history stages is poorly understood in freshwater invertebrates, but may be equally, if not more, important than later stages in influencing population dynamics and survival. Here, we investigated how exposure of embryos of the pond snail *Lymnaea stagnalis* to predator kairomones altered traits associated with protection (crawl out behaviour and shell size) in hatchling snails. We also investigated whether levels of plasticity were influenced by habitat of origin by comparing reaction norms from sites with different levels of predation risk: three high risk (predatory fish present) and three low risk (predatory fish absent).

2. F2 embryos from snails from each population were exposed to kairomones from a predatory fish (*Tinca tinca*) or control conditions for their entire development up to the point of hatching. Their avoidance behaviour and size (spire height) were then measured 1 day and 7 days after hatching.

3. One day post-hatching, levels of predator avoidance, primarily time spent at the water line, were significantly greater in high-risk versus low-risk populations; however, avoidance was also increased in hatchling snails exposed to predator cues as embryos, compared with those developing in control conditions. Snails exposed to predator cues during development were smaller, and there was a strong negative relationship between size and avoidance behaviour, suggesting a trade-off or trait compensation between growth and behaviour.

4. At 7 days post-hatching, a similar pattern in avoidance behaviour was found, with increased avoidance in snails from high-risk habitats and in those exposed to predator kairomones during their development. The primary avoidance response switched to crawling above the waterline at 7 days post-hatching. There was no significant difference in shell size between treatments, suggesting that predator-exposed snails had exhibited 'catch-up' growth. This enhanced investment in growth between 1 day and 7 days post-hatching was positively correlated with elevated avoidance behaviour across treatment groups, demonstrating that snails switched to trait co-specialisation of defence traits.

5. During the first few days post-hatching, snails alter the type of avoidance behaviour and also the relationship between morphological and behavioural defence traits. The degree of expression of these defensive traits in hatchling snails is influenced by both their developmental environment and local adaptation to their predation habitat that may have large implications for survival.

Keywords: developmental plasticity, embryo, local adaptation, *Lymnaea stagnalis*, predator

Introduction

Failure to respond to the presence of predators is likely to result in mortality or injury, and freshwater taxa exhibit a wide range of defensive mechanisms to reduce the probability of both of these scenarios, including altered life histories, avoidance behaviours, morphological and chemical defences and size refugia (Ferrari,

Correspondence: Sarah Dalesman, Institute of Biological, Environmental and Rural Sciences, IBERS New building 0.18, Aberystwyth University, Penglais, Aberystwyth, Ceredigion. SY23 3DA, U.K. E-mail: sad31@aber.ac.uk

Wisenden & Chivers, 2010). However, investment in defensive traits such as avoidance behaviour and morphological defences is costly, and potential trade-offs may mean that species invest in one main form of defence over another (Rundle & Brönmark, 2001). At the same time, prey should aim to express trait levels that match risk levels in their environment through phenotypic plasticity (Relyea, 2002; Hammill, Rogers & Beckerman, 2008), although the degree to which such induced defences can be expressed may be limited by other environmental factors (Hansson, 2000; Rundle *et al*., 2004). Whilst there has been recent interest in investigating the biology of such induced defences in aquatic organisms, information on the degree to which such plasticity affects embryos and early life stages is scarce. However, responses to predation threat early in ontogeny may be of equal if not greater importance, either acting directly as the target of selection or influencing the fitness of later life stages.

Much of what we do know about induced defences in early life stages concerns vertebrates. In amphibians, embryonic exposure to predation cues altered developmental rates, growth and survival (Orizaola & Brana, 2005; Vonesh & Bolker, 2005), as well as post-hatching antipredator behaviour (Mathis *et al*., 2008; Ferrari & Chivers, 2009). A similar enhancement in antipredator behaviour was demonstrated in convict cichlids, *Amatitlania siquia*, following exposure to predation-related cues in the egg (Nelson, Alemadi & Wisenden, 2013). There is some evidence that invertebrates may also respond to predator cues early in ontogeny. For example, Laforsch & Tollrian (2004) showed that two *Daphnia* species initiated the growth of morphological defences as embryos in response to predator kairomones, and Rundle *et al*. (2011) showed that embryos of the pond snail *Radix balthica* showed an earlier onset of crawling within the egg capsule when exposed to kairomones, which was hypothesised might be linked to an enhanced crawling ability in hatchlings.

In addition to the ability to adjust traits following experience of predation threat, local adaptation of antipredator traits is common among freshwater species. The introduction of predators to freshwater habitats has been shown to lead to the rapid evolution of avoidance behaviours in Trinidadian guppies (*Poecilia reticulata*: O'steen, Cullum & Bennett, 2002) and *Daphnia magna* (Cousyn *et al*., 2001). Differences in predation threat between habitats have also been shown to be associated with differential levels of avoidance behaviours in anurans (Laurila, 2000), amphipods (Åbjörnsson, Hansson & Brönmark, 2004) and gastropods (Dalesman, Rundle & Cotton, 2007b) and of morphological (i.e. shell) defences in gastropods (Brönmark, Lakowitz & Hollander, 2011; Ahlgren *et al*., 2014). Plasticity in antipredator traits may also relate to predator regime, where prey demonstrate a more pronounced induced antipredator response following experience of predation threat when they naturally overlap with the predator, as shown in Trinidadian guppies and three-spine sticklebacks, *Gasterosteus aculeatus* (reviewed in Kelley & Magurran, 2003). As yet, however, we are not aware of any investigation of population-level variation in developmental plasticity in response to predation threat in embryos of aquatic species.

Here, we investigated whether early (i.e. embryonic) exposure to predator kairomones influenced induced defences and, if so, whether the degree of plasticity was influenced by local adaptation. We used the gastropod *Lymnaea stagnalis* (L.) as a model. This species is known to adjust its behaviour in line with the degree of predation threat (Dalesman *et al*., 2006; Dalesman, Rundle & Cotton, 2009b; Dalesman & Rundle, 2010) and also exhibits local adaptation to predation-related cues (Dalesman *et al*., 2007a,b). In addition to behavioural avoidance, invertebrate species often demonstrate induced morphological defences following exposure to predators, which appear to be related to outgrowing predator gape width or enhancement of protective structures (plankton; Lass & Spaak, 2003; gastropods; Covich, 2010; crustaceans; Hazlett, 2011). Therefore, we aimed to assess whether gastropod hatchlings exhibit both behavioural and morphological defence traits following embryonic exposure to predation threat.

We exposed *L. stagnalis* from six populations experiencing different predator regimes, three high-risk (i.e. overlapping with fish predators) and three low-risk populations (invertebrate predators only) (Dalesman *et al*., 2007b) to kairomones from tench (*Tinca tinca* L.) during their embryonic development. We then assessed antipredator behaviour and shell morphology (spire height) 1 day and 7 days post-hatching to determine whether hatchlings demonstrated induced defences following embryonic exposure. We predicted that those snails exposed to predator kairomones during their embryonic development would demonstrate enhanced antipredator traits and that trait plasticity would be greatest in populations that originated from habitats containing predatory fish.

Methods

Adult great pond snails, *Lymnaea stagnalis*, were collected in September 2012 from six sites on the Somerset levels,

U.K., three large river sites containing predatory fish including tench (P1, P3 & P5) and three small drainage ditches lacking predatory fish (P2, P4 & P6). These sites had been previously identified as sites where *L. stagnalis* juvenile antipredator behaviour is locally adapted to predation regime (see Dalesman *et al.*, 2007b) and to our knowledge had experienced stable predation regimes for at least 8 years. They are also located in a paired fashion, such that paired ditch and river sites are 500–900 m apart, but each pair of sites is >5 km apart to account for geographical variation. Pairs of sites were P1 & P2, P3 & P4 and P5 & P6. On return to the laboratory, animals were maintained at 20 ± 1 °C under a 14 : 10 light:dark regime at a stocking density of 2 snails per litre in aerated artificial pond water (PW) with 80 mg L^{-1} [Ca^{2+}] – a concentration of calcium at which shell growth is not limited and is similar to those at the sites from which the experimental populations were collected (Dalesman & Lukowiak, 2010). Snails were fed *ad libitum* on lettuce supplemented with trout pellets (Efico Alpha 790, Biomar, Brande, Denmark). F1 adults were reared in the laboratory from wild-caught F0 adults ($N \geq 50$ per population), with a minimum of 50 adults maintained to contribute to the F1 generation to prevent inbreeding. F2 embryos from these laboratory-reared adults were then used to conduct the experiments.

Tench, *Tinca tinca*, were used to produce predatory fish kairomones as this fish species is known to be a molluscivore (Brönmark, 1994) and is present at the large canal sites, and juvenile *L. stagnalis* from all sites demonstrate behavioural avoidance in response to cues associated with predation threat from this species (Dalesman *et al.*, 2007b). Ten tench (10 ± 1 cm) were obtained from The Carp Company (Bourne Valley Fish Farm, Hamptons Road, Hadlow, Tonbridge, Kent, U.K.). They were maintained in glass aquaria 600 mm × 300 mm × 300 mm in 47 L artificial pond water at a density of 1 fish per 4.7 L at 20 °C on the 14 : 10 full-spectrum light : dark regime. Water in the home aquaria was on constant flow-through and aerated throughout. The tank base was covered in gravel, and shelter provided in the form of opaque UPVC drainage pipes and artificial pond weed. The tench were fed on chironomid larvae to satiation once per day throughout the experiment and hence avoided the potential for dietary cues to affect the trials. To produce kairomones, 3 tench were randomly selected from the laboratory population and held in 4 l of static aerated PW for 1 h (Dalesman *et al.*, 2006). Fresh kairomone water was produced for each exposure.

Freshly laid egg masses were collected from the aquaria daily. Egg masses were split open using watchmaker forceps, and eggs removed and placed in individual cell wells (diameter 1 cm, depth 2 cm, 24 per plate: Nunc®, NalgeNunc International, New York, U.S.A.) containing either predator kairomone or control water (PW alone) in a randomised block design, such that equal numbers of eggs from each egg mass were exposed to each treatment group. Replicates from each egg mass were exposed in each treatment group to control for any developmental mortality; however, a single individual randomly selected from each egg mass was assessed for their behavioural response within each treatment group to avoid pseudoreplication among full sibs. Kairomone pond water or PW was replaced three times per week for the duration of embryonic development (14 ± 2 days). Exposure to kairomones was ceased on the day of hatching, and hatchlings were transferred into new cell well plates, 1 hatchling per well in 1 ml PW, and provided with lettuce *ad libitum*.

Morphology

To assess growth using the spire height of hatchlings (distance from the outer edge of the aperture to the apex of the spire), a randomly selected subset of individuals from each developmental exposure group were euthanised immediately following each behavioural trial, that is at 1 day and 7 days post-hatching, using submersion in ethanol. Images were taken using a Nikon-SMZ 1500 microscope with an attached WD 54 Nikon JAPAN HR Plan Apo camera and NIS-Elements imaging software (Nikon Instruments Europe B.V., Amsterdam, Netherlands). These images were then used to measure spire height using the KLONK image analysis package (KLONK, Ringsted, Denmark). Spire height data, either 1 day (N = 11–20) or 7 days (N = 10–17) post-hatching, were analysed using ANOVA in SPSS 17.0 (SPSS Inc., Chicago, IL, U.S.A.) with habitat type (river vs. ditch) and developmental exposure (control versus tench) as fixed factors and population nested in habitat type (P1–P6) as a random factor in the analyses.

Behaviour

A common-garden approach was used to assess antipredator behaviour. All hatchling snails were maintained in PW and then tested either 1 day or 7 days post-hatching. In those maintained for 7 days, water was changed three times during this period providing fresh PW. One hour prior to testing, both the lettuce and 0.5 mL of

water were removed from each cell well, leaving the hatchling in 0.5 mL of PW to acclimate. During each behavioural trial, half the snails pre-exposed to each treatment group (predator kairomones or control PW) were exposed to kairomones and half were exposed to control PW only in an orthogonal design. At the start of each behavioural trial, 0.5 mL of either kairomone PW or control PW was added to each well, and the position of each snail was recorded immediately. The position of each individual was then subsequently recorded every 5 min for 1 h (total of 13 recordings). The data were then separated into time spent on the base and sides of the cell well (not considered avoidance behaviour), and time spent in avoidance, either at the water's surface or crawled above the water line, and the proportion of time spent in each of these positions throughout the duration of the experiment was calculated.

Behavioural data were analysed using three responses that were considered possible antipredator responses: time at the water surface, time spent crawled out and a combination of both times considered avoidance behaviours (Dalesman *et al.*, 2006). Effect size data were used for analyses and were calculated for each treatment by subtracting the average response under control conditions from each of the individual response replicate values for tench kairomones. Data comparing how they responded either 1 day (N = 14–36) or 7 days (N = 15–27) post-hatching were analysed using analysis of variance (ANOVA) in SPSS using the reaction norm for time spent at the surface, time spent crawled out and both of these measures combined (total avoidance) as the response variables. Fixed factors included in the analyses were habitat type (ditch versus river) and pre-exposure (exposure to control versus tench kairomones during development), and population nested in habitat type (six populations, three per habitat) was included as a random factor.

Covariance of defence traits

To assess whether snails exhibited trait compensation or co-specialisation, we assessed the relationship between the mean population response in morphological defence (spire height) and the mean population response in behavioural avoidance to tench kairomones within pre-exposure groups (control versus tench). Mean population response data were analysed using Pearson's correlation in SPSS using spire height at day 1, spire height at day 7 and also growth between day 1 and 7 (mean spire height on day 7 minus mean spire height on day 1).

Results

One day post-hatching

One-day-old hatchling snails exposed to tench kairomones had significantly lower spire heights compared with control snails (Fig. 1a; $F_{1,4.077} = 19.74$, $P = 0.011$). Those from high-risk populations showed a marginally non-significant trend towards being smaller than those from low-risk populations (Fig. 1a; $F_{1,4.030} = 7.50$, $P = 0.052$). Individual populations (within habitat) did not differ in spire height. Size differences were independent of developmental rate, as exposure to kairomones, habitat and population did not affect development time.

One day after hatching, snails exposed to tench kairomones during development had significantly greater total avoidance behaviour (Fig. 2c; $F_{1,11.850} = 65.79$, $P < 0.001$) and spent longer at the water line (Fig. 2a; $F_{1,8.534} = 27.11$, $P = 0.001$) than those reared under

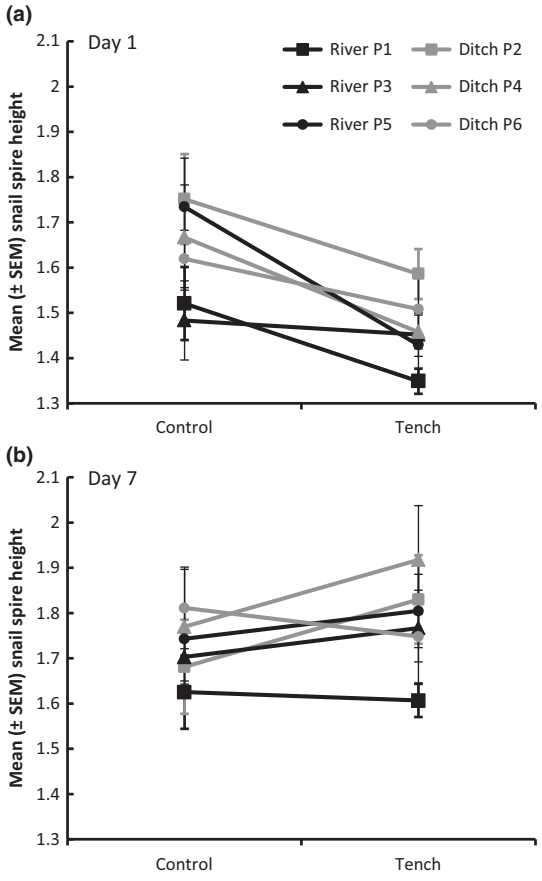

Fig. 1 Spire height of *Lymnaea stagnalis* hatchlings 1 day (a) and 7 days (b) post-hatching following exposure to control conditions or tench kairomone pond water during embryonic development. Values are means (± SEM).

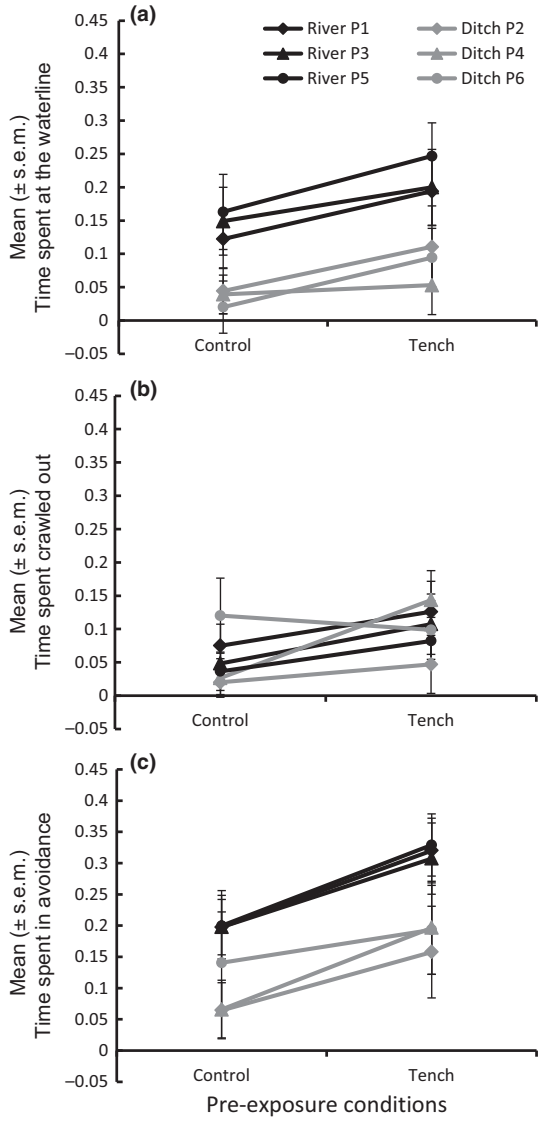

Fig. 2 Reaction norms for behavioural avoidance effect sizes 1 day post-hatching in *Lymnaea stagnalis* individuals reared in the absence or presence of tench kairomones. Behavioural measures are time spent at the water line (a), time spent crawled out of the water (b) and total time spent in avoidance (c). Values are means (± SEM).

control conditions, and there was a marginally non-significant trend towards increased time spent crawled out (Fig. 2b; $F_{1,4.769} = 6.616$, $P = 0.052$). Hatchlings from high-risk habitats also exhibited significantly higher total avoidance (Fig. 2c; $F_{1,6.762} = 47.61$, $P < 0.001$) and spent significantly longer at the water line (Fig. 2a; $F_{1,5.215} = 41.67$, $P = 0.001$) than those from low-risk habitats. However, habitat of origin did not significantly affect how snails responded to developmental exposure to predator kairomones. Individual population had no effect on any of the avoidance behaviours measured.

Seven days post-hatching

Spire height did not differ among groups 7 days post-hatching dependant on developmental exposure group, habitat or population (Fig. 1b).

Seven days after hatching, snails exposed to kairomones during development exhibited a significantly higher total avoidance behaviour (Fig. 3c; $F_{1,4.240} = 11.26$, $P = 0.026$) and time spent crawled out of the water (Fig. 3b; $F_{1,4.165} = 12.90$, $P = 0.021$), but there was no significant effect on time spent at the water line. Habitat of origin did not alter the time spent at the water line

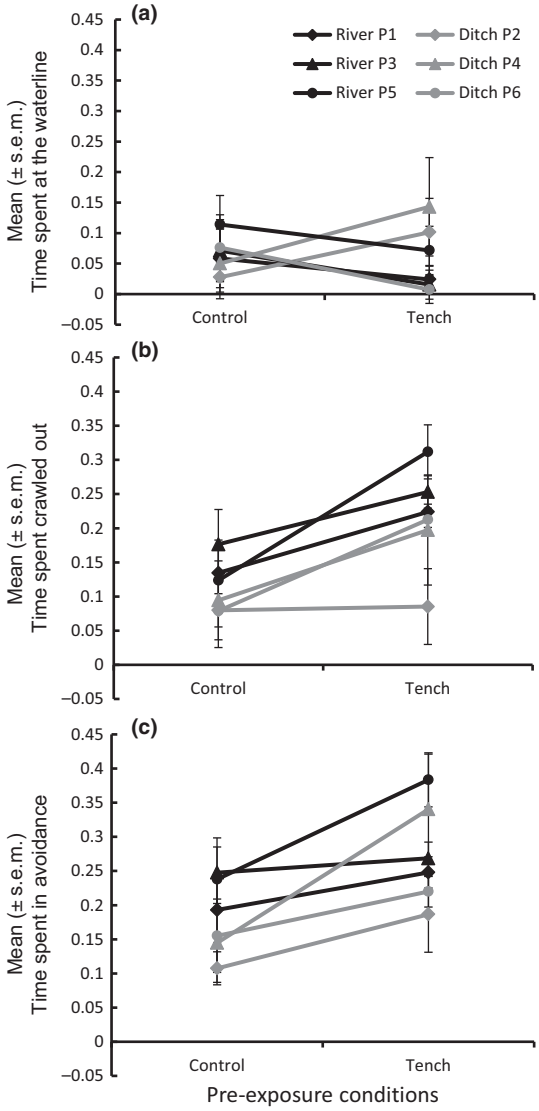

Fig. 3 Reaction norms for behavioural avoidance effect sizes 7 days post-hatching in *Lymnaea stagnalis* individuals reared in the absence or presence of tench kairomones. Behavioural measures are time spent at the water line (a), time spent crawled out of the water (b) and total time spent in avoidance (c). Values are means (± SEM).

(Fig. 3a) or total avoidance (Fig. 3c), but snails from high-risk habitats did spend significantly longer crawled out of the water compared with low-risk habitats (Fig. 3b: time spent crawled out: $F_{1,4.194} = 9.62$, $P = 0.034$). Similar to behavioural responses 1 day post-hatching, the effect of developmental exposure to predator kairomones on behavioural avoidance was not dependant on the habitat of origin. Population of origin within habitat types did not alter any of the avoidance behaviours measured.

Covariance of defence traits

One day post-hatching, there was a significant negative correlation between spire height and total time spent in avoidance (Fig. 4a; $r = -0.82$, $P = 0.043$) for snails exposed to predator cues during their development. Snails exposed to control conditions during development demonstrated no significant relationship between spire height and avoidance behaviour (Fig. 4a; $r = -0.57$, $P = 0.239$). Hence, it appeared that there was a potential trade-off between investment in growth and investment in behavioural avoidance that was associated with embryonic plasticity. Spire height and avoidance behaviour did not covary in 7-day-old hatchlings, irrespective of whether they were exposed to tench kairomones ($r = 0.36$, $P = 0.484$) or control conditions ($r = -0.12$, $P = 0.815$) during embryonic development (Fig. 4b). However, when assessed by average increase in spire height between day 1 and day 7, there was a strong positive relationship between shell growth and avoidance behaviour in snails exposed to tench kairomones during embryonic development (Fig. 4c; $r = 0.86$, $P = 0.029$), but not in snails exposed to control conditions as embryos ($r = 0.309$, $P = 0.551$). This suggests that rather than trait compensation shown 1 day post-hatching, 7-day-old snails exposed to kairomones during development demonstrate trait covariation in investment in behavioural avoidance and shell growth (morphological defence).

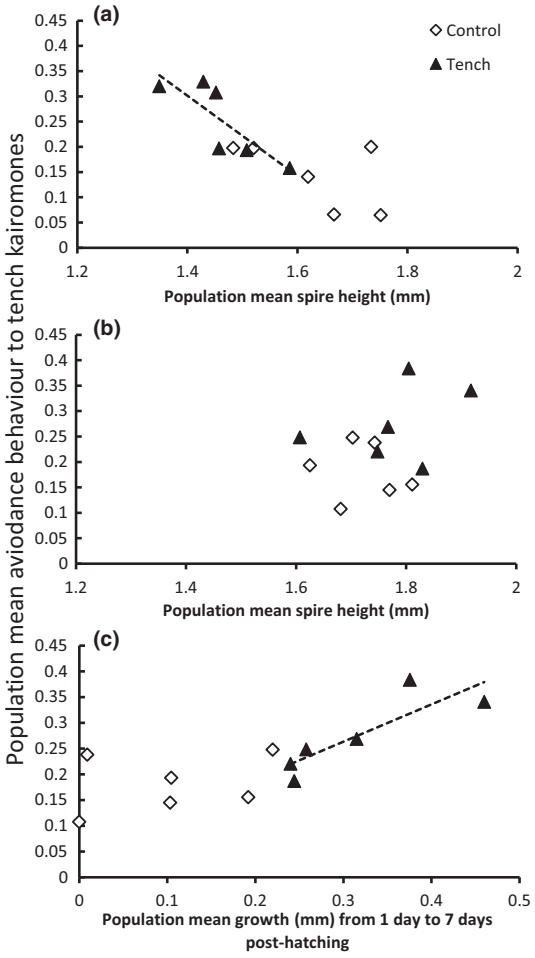

Fig. 4 Relationship between spire height or growth and total time spent in avoidance behaviour in response to tench kairomones following embryonic development in the absence (white diamonds) or presence (black triangles) of tench kairomones. Panels show relationships between (a) – mean population spire height and mean total time spent in avoidance 1 day post-hatching; (b) – mean population spire height and mean total time spent in avoidance 7 days post-hatching; and (c) – mean population shell growth between 1 and 7 days and mean total avoidance behaviour 7 days post-hatching. Trend lines (for a & c) show significant Pearson's correlations for snails exposed to tench kairomones during their development.

Discussion

Here, we have shown that hatchling *Lymnaea stagnalis* show avoidance behaviours that are influenced by both their developmental environment and the local habitat from which their grandparents originated. Snails exposed to predator kairomones during development exhibited significantly higher levels of avoidance behaviour than control snails, and avoidance was also significantly higher in snails from high-risk versus low-risk habitats. In contrast, snails had a smaller shell size 1 day post-hatching in high-risk compared with low-risk populations and also demonstrated a significantly smaller size following predator cue exposure, suggesting a trade-off or trait compensation (Rundle & Brönmark, 2001) between growth and behaviour immediately post-hatching. This trade-off between behavioural and morphological defence traits in 1-day-old snails was substantiated by a strong ($r = -0.82$) negative relationship between avoidance behaviour and shell spire height in snails exposed to predator cues during development. At 7 days post-hatching, total avoidance behaviour

remained significantly higher in snails that had experienced predator kairomones during development, and there was also a significantly higher crawl out response in snails from high-risk versus low-risk populations. However, shell spire height did not vary among treatment groups, which suggests that predation-exposed snails had exhibited 'catch-up' growth. The increase in average spire height we observed between 1-day- and 7-day-old snails was positively correlated with avoidance behaviour in snails exposed to predator cues as embryos, supporting the inference that by 7 days post-hatching juvenile *L. stagnalis* exhibit cospecialisation in defence traits (Dewitt, Sih & Hucko, 1999), where predator-exposed snails display both enhanced investment in morphological and behavioural defences relative to control-exposed animals.

The enhanced antipredator behaviour post-hatching confirms that embryos of invertebrates such as gastropods are able to detect and respond to predation threat in their environment as do embryonic anurans (Mathis *et al.*, 2008, Ferrari, Manek & Chivers, 2010) and fish (Nelson *et al.*, 2013). The slopes of reaction norms for total avoidance one day after hatching were very similar across populations, suggesting that the level of plastic response for behaviour was consistent and particularly so for high-risk populations. A similar consistency in reaction norm slopes was found for morphological plasticity in the juvenile *Radix balthica* populations exposed to predator kairomones (Brönmark *et al.*, 2011). Although the significant difference between high- and low-risk populations in our study persisted at day seven, the variation among reaction norms was much greater. This indicates that the influence from the developmental environment changes over time and may be related to the switch in avoidance behaviour. Crawl out behaviour, which is only apparent in 7-day-old juveniles, includes a risk of desiccation that needs to be balanced with both perceived predation threat and also resistance to desiccation which may relate to shell size (Mcquaid, 1982). The positive relationship between avoidance behaviour and shell growth (but not overall size) in 7-day-old juveniles indicates that this relationship may be complex, resulting in the variability in reaction norms found here.

The reduced growth rate in embryos exposed to predator kairomones is surprising given that previous studies have shown that juvenile and adult gastropods increase their growth rates and shell size in response to predation threat (Crowl & Covich, 1990; Rundle *et al.*, 2004; Hoverman & Relyea, 2007; Auld & Relyea, 2011). It suggests that snail embryos may show a very different response to juveniles and adult snails and could reflect some level of prenatal stress similar to that shown to decrease birth or hatching size in vertebrates, including humans (McCormick, 1999; Love & Williams, 2008; Harris & Seckl, 2011). Isolation of individual eggs from the egg mass has been found to result in synchronous hatching in *L. stagnalis* embryos (Marois & Croll, 1991), and we found no effect of cue exposure on hatching time. Miner, Donovan & Andrews (2010) showed that cues from a predatory crab delayed hatching in the marine gastropod *Nucella lamellose*, but found no effect of predator exposure on hatchling size or subsequent growth. However, marine mud snails, *Ilyanassa obsolete*, demonstrated no significant alteration in hatching time, similar to our study, but did demonstrate an increase in hatchling size in response to predation threat (Schwab & Allen, 2014). This increase in hatchling size was dependant on predator identity, with a significant increase in response to green crab, *Carcinus maenus*, kairomones, but not those from sea urchins, *Strongylocentrus droebachiensis*. Therefore, the decrease in hatchling size we found in *L. stagnalis* may be a predator-specific response to *T. tinca* kairomones, rather than a generalised response to all predators. Finally, Vaughn (2007) demonstrated that veliger larvae of the marine gastropod *Littorina scutulata* developed shells with smaller apertures and a rounder shape when exposed to kairomones from predatory crab larvae. She also demonstrated that this morphology gave protection from predation by crab larvae. A similar investigation of the details of shell plasticity and its adaptive value in hatchling *Lymnaea stagnalis* would be instructive.

The apparent trade-off we observed between growth (and potential protection from a larger shell) and enhanced antipredator behaviour in predator-exposed individuals 1 day post-hatching suggests a form of trait compensation, where individuals with weaker morphological defences exhibit the strongest behavioural defences (Rundle & Brönmark, 2001; Rundle *et al.*, 2004). Despite their smaller size on hatching, however, after 7 days, there was no longer a significant difference in spire height between snails exposed to predator kairomones during development and control animals. This suggests that kairomone-exposed snails showed some form of 'catch-up' growth, similar to that shown in *Helisoma trivolis* (Hoverman & Relyea, 2007). For *Helisoma*, however, there were costs associated with a late induction of higher shell growth. In our study, the fact that predator-exposed individuals also continued to demonstrate increased antipredator behaviour suggests that there was no immediate cost of this accelerated shell

growth. Such cospecialisation of morphological and behavioural avoidance defensive traits has been demonstrated previously in a freshwater gastropod (Dewitt *et al*., 1999) and, in *Lymnaea stagnalis*, may be an optimal strategy until shell defences have developed to a degree that behavioural defences are less important (see Rundle & Brönmark, 2001).

Calcium availability provided during the experiment may have played an important factor in enabling *L. stagnalis* to exhibit this catch-up growth during the early post-hatching phase. Restricting calcium availability has been shown to have negative effects on both growth and behaviour in *L. stagnalis*. Reducing calcium levels from 90 to 45 mg L^{-1} prevents older juvenile snails from exhibiting predator-induced shell thickening (Rundle *et al*., 2004), and lower levels (20 mg L^{-1}) increased metabolic rate and reduced activity levels in adults (Dalesman & Lukowiak, 2010). Previous work in a closed system with *H. trivolis* suggested that a decline in calcium levels over time may have restricted the ability of *H. trivolis* to increase shell thickness in response to predation threat (Hoverman & Relyea, 2007). In our study, the regular water changes during the experiment will have maintained calcium levels close to the original concentration throughout. The level of calcium provided during this study (80 mg L^{-1}) approximates the average calcium availability experienced by snails from our collection sites on the Somerset levels (82 ± 7.4 mg L^{-1}), reflecting natural conditions experienced by the populations in this study.

There were clear differences between snails from low- and high-risk habitats in terms of the degree of avoidance behaviour they showed in response to predator kairomones. Snails from high-risk populations spent longer demonstrating antipredator behaviour than those from low-risk populations. This local adaptation is consistent with that observed for juvenile (~1 month old) *L. stagnalis* reared from adults collected at the same field sites used in the current study (Dalesman *et al*., 2007b); F1 juveniles demonstrated a stronger innate response to tench kairomones when their parents overlapped in distribution with this fish. Hence, these innate differences in antipredator behaviour are present throughout early development, from hatchling to the advanced juvenile stage. These differences also demonstrate stability over prolonged periods within populations, as found in the eight-year gap between this study and the previous one on juvenile snails (Dalesman *et al*., 2007b). It is possible that transgenerational effects are present, as the grandparents of exposed embryos were wild caught and so experienced different predation regimes (Agrawal, Laforsch & Tollrian, 1999; Moran, Dias & Marshall, 2010). However, selection on antipredator behaviour in the laboratory indicates a strong genetic element to this behaviour (Dalesman, Rundle & Cotton, 2009a). Possible local adaptation in morphological traits relating to defence has also been shown in freshwater gastropods, with shells of *Radix balthica* differing in their shape between fishless ponds and those containing fish in southern Sweden following rearing under common-garden conditions (Brönmark *et al*., 2011).

Contrary to one of our original predictions, the degree of plasticity in avoidance behaviour was indistinguishable between high-risk populations and low-risk populations following embryonic exposure to predator cues. This result is supported by previous work demonstrating that a more prolonged exposure to predator cues, throughout embryonic development through to four weeks post-hatching (spire height 6 mm), completely compensates for innate differences in avoidance behaviour among strains (Dalesman *et al*., 2009b). What our data suggest is that very early life experience during embryonic development may play an integral part in the snail's ability to respond to predation threat where innate responses have been diminished through local adaptation (Dalesman *et al*., 2007b); in this case, the fact that snails originating from sites of low predation risk showed an equal degree of behavioural plasticity to those from high-risk populations. Embryonic experience may therefore be extremely important in allowing populations to persist in a changing predation environment. At the same time, the alteration in the type of avoidance response shown and a switch from trait compensation to trait cospecialisation in snails exposed to predator kairomones suggests that hatchling snails exhibit subtle changes in their investment strategies that may enable them to better survive during this early stage of their post-hatching development.

Acknowledgments

S.D. is supported by a Leverhulme Trust Early Career Fellowship, and the Association for the Study of Animal Behaviour provided an undergraduate project scholarship to A.T.

References

Åbjörnsson K., Hansson L.A. & Brönmark C. (2004) Responses of prey from habitats with different predator regimes: local adaptation and heritability. *Ecology*, **85**, 1859–1866.

Agrawal A.A., Laforsch C. & Tollrian R. (1999) Transgenerational induction of defences in animals and plants. *Nature*, **401**, 60–63.

Ahlgren J., Yang X., Hansson L.A. & Brönmark C. (2014) Camouflaged or tanned: plasticity in freshwater snail pigmentation. *Biology Letters*, **9**, 20130464.

Auld J.R. & Relyea R.A. (2011) Adaptive plasticity in predator-induced defenses in a common freshwater snail: altered selection and mode of predation due to prey phenotype. *Evolutionary Ecology*, **25**, 189–202.

Brönmark C. (1994) Effects of tench and perch on interactions in a fresh-water, benthic food-chain. *Ecology*, **75**, 1818–1828.

Brönmark C., Lakowitz T. & Hollander J. (2011) Predator-induced morphological plasticity across local populations of a freshwater snail. *PLoS ONE*, **6**, e21773.

Cousyn C., De Meester L., Colbourne J.K., Brendonck L., Verschuren D. & Volckaert F. (2001) Rapid, local adaptation of zooplankton behavior to changes in predation pressure in the absence of neutral genetic changes. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 6256–6260.

Covich A.P. (2010) Winning the biodiversity arms race among freshwater gastropods: competition and coexistence through shell variability and predator avoidance. *Hydrobiologia*, **653**, 191–215.

Crowl T.A. & Covich A.P. (1990) Predator-induced life-history shifts in a fresh-water snail. *Science*, **247**, 949–951.

Dalesman S. & Lukowiak K. (2010) Effect of acute exposure to low environmental calcium alters respiration and locomotion of *Lymnaea stagnalis* (L.). *Journal of Experimental Biology*, **213**, 1471–1476.

Dalesman S. & Rundle S.D. (2010) Cohabitation enhances the avoidance response to heterospecific alarm cues in a freshwater snail. *Animal Behaviour*, **79**, 173–177.

Dalesman S., Rundle S.D., Bilton D.T. & Cotton P.A. (2007a) Phylogenetic relatedness and ecological interactions determine anti-predator behaviour. *Ecology*, **88**, 2462–2467.

Dalesman S., Rundle S.D., Coleman R.A. & Cotton P.A. (2006) Cue association and antipredator behaviour in a pulmonate snail, *Lymnaea stagnalis*. *Animal Behaviour*, **71**, 789–797.

Dalesman S., Rundle S.D. & Cotton P.A. (2007b) Predator regime influences innate anti-predator behaviour in the freshwater gastropod *Lymnaea stagnalis*. *Freshwater Biology*, **52**, 2134–2140.

Dalesman S., Rundle S.D. & Cotton P.A. (2009a) Crawl-out behaviour in response to predation cues in an aquatic gastropod: insights from artificial selection. *Journal of Evolutionary Ecology*, **23**, 907–918.

Dalesman S., Rundle S.D. & Cotton P.A. (2009b) Developmental plasticity compensates for selected low levels of behavioural avoidance in a freshwater snail. *Animal Behaviour*, **78**, 987–991.

Dewitt T.J., Sih A. & Hucko J.A. (1999) Trait compensation and cospecialization in a freshwater snail: size, shape and antipredator behaviour. *Animal Behaviour*, **58**, 397–407.

Ferrari M.C.O. & Chivers D.P. (2009) Sophisticated early life lessons: threat-sensitive generalization of predator recognition by embryonic amphibians. *Behavioral Ecology*, **20**, 1295–1298.

Ferrari M.C.O., Manek A.K. & Chivers D.P. (2010) Temporal learning of predation risk by embryonic amphibians. *Biology Letters*, **6**, 308–310.

Ferrari M.C.O., Wisenden B.D. & Chivers D.P. (2010) Chemical ecology of predator-prey interactions in aquatic ecosystems: a review and prospectus. *Canadian Journal of Zoology-Revue Canadienne De Zoologie*, **88**, 698–724.

Hammill E., Rogers A. & Beckerman A.P. (2008) Costs, benefits and the evolution of inducible defences: a case study with *Daphnia pulex*. *Journal of Evolutionary Biology*, **21**, 705–715.

Hansson L.A. (2000) Induced pigmentation in zooplankton: a trade-off between threats from predation and ultraviolet radiation. *Proceedings of the Royal Society B: Biological Sciences*, **267**, 2327–2331.

Harris A. & Seckl J. (2011) Glucocorticoids, prenatal stress and the programming of disease. *Hormones and Behavior*, **59**, 279–289.

Hazlett B.A. (2011) Chemical cues and reducing the risk of predation. In: *Chemical Communication in Crustaceans* (Ed. T. Breithaupt), pp. 355–370. Springer, New York.

Hoverman J.T. & Relyea R.A. (2007) How flexible is phenotypic plasticity? Developmental windows for trait induction and reversal. *Ecology*, **88**, 693–705.

Kelley J.L. & Magurran A.E. (2003) Learned predator recognition and antipredator responses in fishes. *Fish and Fisheries*, **4**, 216–226.

Laforsch C. & Tollrian R. (2004) Embryological aspects of inducible morphological defenses in *Daphnia*. *Journal of Morphology*, **262**, 701–707.

Lass S. & Spaak P. (2003) Chemically induced anti-predator defences in plankton: a review. *Hydrobiologia*, **491**, 221–239.

Laurila A. (2000) Behavioural responses to predator chemical cues and local variation in antipredator performance in *Rana temporaria* tadpoles. *Oikos*, **88**, 159–168.

Love O.P. & Williams T.D. (2008) The adaptive value of stress-induced phenotypes: effects of maternally derived corticosterone on sex-biased investment, cost of reproduction, and maternal fitness. *American Naturalist*, **172**, E135–E149.

Marois R. & Croll R.P. (1991) Hatching asynchrony within the egg mass of the pond snail, *Lymnaea stagnalis*. *Invertebrate Reproduction & Development*, **19**, 139–146.

Mathis A., Ferrari M.C.O., Windel N., Messier F. & Chivers D.P. (2008) Learning by embryos and the ghost of predation future. *Proceedings of the Royal Society B-Biological Sciences*, **275**, 2603–2607.

McCormick M.I. (1999) Experimental test of the effect of maternal hormones on larval quality of a coral reef fish. *Oecologia*, **118**, 412–422.

Mcquaid C.D. (1982) The influence of desiccation and predation on vertical size gradients in populations of the gastropod *Oxystele variegata* (Anton) on an exposed rocky shore. *Oecologia*, **53**, 123–127.

Miner B.G., Donovan D.A. & Andrews K.E. (2010) Should I stay or should I go: predator- and conspecific-induced hatching in a marine snail. *Oecologia*, **163**, 69–78.

Moran D.T., Dias G.M. & Marshall D.J. (2010) Associated costs and benefits of a defended phenotype across multiple environments. *Functional Ecology*, **24**, 1299–1305.

Nelson A.B., Alemadi S.D. & Wisenden B.D. (2013) Learned recognition of novel predator odour by convict cichlid embryos. *Behavioral Ecology and Sociobiology*, **67**, 1269–1273.

Orizaola G. & Brana F. (2005) Plasticity in newt metamorphosis: the effect of predation at embryonic and larval stages. *Freshwater Biology*, **50**, 438–446.

O'steen S., Cullum A.J. & Bennett A.F. (2002) Rapid evolution of escape ability in Trinidadian guppies (*Poecilia reticulata*). *Evolution*, **56**, 776–784.

Relyea R.A. (2002) Costs of phenotypic plasticity. *American Naturalist*, **159**, 272–282.

Rundle S.D. & Brönmark C. (2001) Inter- and intraspecific trait compensation of defence mechanisms in freshwater snails. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **268**, 1463–1468.

Rundle S.D., Smirthwaite J.J., Colbert M.W. & Spicer J.I. (2011) Predator cues alter the timing of developmental events in gastropod embryos. *Biology Letters*, **7**, 285–287.

Rundle S.D., Spicer J.I., Coleman R.A., Vosper J. & Soane J. (2004) Environmental calcium modifies induced defences in snails. *Proceedings of the Royal Society of London Series B-Biological Sciences*, **271**, S67–S70.

Schwab D.B. & Allen J.D. (2014) Size-specific maternal effects in response to predator cues in an intertidal snail. *Marine Ecology Progress Series*, **499**, 127–141.

Vaughn D. (2007) Predator-induced morphological defenses in marine zooplankton: a larval case study. *Ecology*, **88**, 1030–1039.

Vonesh J.R. & Bolker B.M. (2005) Compensatory larval responses shift trade-offs associated with predator-induced hatching plasticity. *Ecology*, **86**, 1580–1591.

(Manuscript accepted 31 October 2014)